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Applicant:	Blakesley et al.	Group Art Unit:	1637
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Commissioner for Patents
P.O. Box 1450
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SECOND SUPPLEMENTAL DECLARATION OF DR. WALTER KING PURSUANT TO 37 CFR 1.132

I, Dr. Walter King, declare as follows:

1. Further to my Declaration of March 19, 2009 (mailed March 23, 2009), and my Supplemental Declaration of 14 September 2009 (mailed 6 October 2009), I have recently become the Leader, Diagnostics Initiative for the Consumables Division GE Healthcare. At the time of my Supplemental Declaration of 14 September 2009 (mailed 6 October 2009), I was the R&D Leader of Research & Development at GE Healthcare in Cardiff, Wales, United Kingdom beginning in April 25, 2008. Previously (2006-2008), I was the Vice President of Research & Development at Whatman plc (which became part of the Life Sciences Division of GE Healthcare USA during this time), and I was based in the facility in Sanford, Maine. I am an experienced Ph.D. with an extensive background in Bacteriology & Immunology, Virology, and Microbiology & Urology. Prior to my positions with Whatman plc (now part of the Life Sciences Division of GE Healthcare USA), I had previously (2005-2006) been employed as a Director of the Applications Development Group at Nanosphere, Inc., in Northbrook, IL, where I was in charge of development of In Vitro Diagnostic (IVD) products for genetic and infectious disease testing using single nucleotide polymorphism detection technology in patient samples without amplification. Prior to that, I had been a Senior

Director of Product Development in the Vysis Diagnostic Division and Molecular Oncology Program Director at Abbott Laboratories (2002-2005) in Downers Grove, IL, where I was in charge of development of microarray IVD products for molecular cytogenetics and oncology; and a Director of the Genomic Microarray Platform program (1999-2001; genomic array-based assay products for the detection of amplification and deletions for solid tumors, leukemias, lymphomas and prenatal applications) and a Senior Manager of Assay Development (1995-1998; IVD's for Her-2 detection in breast cancer [PMA approval], multiplex aneuploidy detection in bladder cancer [510K approval], and pre-implantation testing in blastomeres and polar bodies) at Vysis, Inc. in Downers Grove, IL. I was also a Senior Scientist in Assay Development (1986-1995; assay formats and chemistries for an automated clinical analyzer; amplification chemistry for detection of respiratory, gastrointestinal and sexually transmitted disease panels; manual non-isotopic probase-based test for detection of Listeria, Salmonella and E. coli) at Gene-Trak Systems, Inc., in Framingham, MA; and was employed in the Biotechnology Group (1985-1986; development of nucleic acid probe technology for the detection of clinical pathogens) of AMOCO Corporation in Naperville, IL. I have been active in the development of various products, have been listed as an inventor on several patents, and have co-authored over thirty publications. I hold a Bachelor of Arts (1973) from the Department of Bacteriology and Immunology of the University of California in Berkeley, CA. I hold a Ph.D. in Virology (1980) from the University of Chicago in Chicago, IL. I served as a Postdoctoral Fellow in the Department of Medicine at the University of Chicago (1980-1982), where I identified the transforming region in the Epstein-Barr Virus (EBV) genome, and in the Departments of Microbiology and Urology at Columbia University Medical Center in New York, NY (1982-1985), where I identified genes involved in the differentiation of embryonal carcinoma cells. I am a member of the American Association for Cancer Research.

2. The subject application, as currently amended, discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) providing a dry matrix or solid medium, wherein said dry matrix or solid medium further comprises: i) a weak base; ii) a chelating agent; and iii) an anionic surfactant

or an anionic detergent; b) contacting the matrix or solid medium with a sample comprising a host cell containing said plasmid or plasmids; c) releasing said one or more plasmids from said host cell and onto said matrix or solid medium; and d) isolating all or a portion of said plasmid or plasmids from said matrix or solid medium. It also discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) contacting a solid medium with a sample comprising a host cell containing said plasmid, wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; ii) a weak base; iii) a chelating agent; and iv) an anionic surfactant or an anionic detergent; b) releasing the plasmid from the host cell and onto said medium; and c) isolating said plasmid from said medium. It also discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) contacting a solid medium with a sample comprising a host cell containing said plasmid, wherein said solid medium protects against degradation of said plasmid and wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; and ii) a composition sorbed to the polymeric matrix, wherein the composition comprises: a weak base; a chelating agent; and an anionic surfactant or an anionic detergent; b) lysing the host cell; c) releasing the plasmid from the host cell and onto said medium; and d) isolating said plasmid from said medium.

3. As noted previously, for many projects, generation of numerous DNA samples from biological specimens is routine. Traditionally, however, handling and archiving a large collection can become a logistical problem for the laboratory. Previously, there had often been a need to use organic solvents or harsh chemicals or to store samples in a freezer. One solution, used in forensic labs, was the bloodstorage medium FTA® Cards. An FTA® Card could be used ***to store genomic*** DNA in the form of dried spots of human whole blood, the cells of which were lysed on the paper. Before analysis of the captured ***genomic*** DNA, a few washing steps would be used to remove the stabilizing chemicals and cellular inhibitors of

enzymatic reactions, but the ***genomic*** DNA would largely remain on the card. ***Isolated bacterial*** DNAs ***spotted*** on FTA® Cards could be an efficient system for storage and retrieval as well, followed by downstream applications, such as PCR ribotyping. Further, ***purified*** plasmid DNA was spotted on treated paper and recovered. Nevertheless, there was a need for a simplified method of ***isolation and storage*** of ***plasmid*** DNA ***directly from cells*** onto a dry matrix or solid medium ***without prior isolation of the plasmid DNA from the host***, followed by its ***subsequent isolation*** from the matrix or solid medium.

4. The current invention addresses these concerns and many other issues as well, such as protection from degradation.

5. I have reviewed the Office Action (mailed 26 January 2010) and the previously filed Response (mailed 6 October 2009) and my previously filed Supplemental Declaration (14 September 2009; mailed 6 October 2009; hereinafter "First Supplemental Declaration" to distinguish it from the present Second Supplemental Declaration).

6. As discussed in my previously filed First Supplemental Declaration, Rogers & Burgoyne describes experiments with genomic DNA. Although Kahn mentions how plasmid DNA can be separated from genomic DNA on the basis of its smaller size or its unique properties of it being a covalently closed circular DNA or, in other words, less complex than genomic DNA, this reference actually teaches away from the present invention. For example, as previously noted, plasmid DNA behaves differently from genomic DNA based on its composition and its structure. It would not be expected that less complex DNA would interact with a solid matrix in the same manner as genomic DNA, so it would not be intuitive that plasmid DNA could be isolated on a solid matrix. In point of fact, prior separation of plasmid DNA from genomic DNA is preferred, as shown in Old & Primrose and other references previously submitted. Burgoyne, however, only demonstrates DNA isolation directly from cells for genomic DNA, whereas the isolation of plasmid DNA requires pre-purification prior to contact with the solid medium. It would not have been at all clear, much

less a matter of reasonable expectation, that isolation of plasmid DNA directly from cells (despite the presence of genomic DNA) would be possible, particularly given the low copy numbers of some plasmids (see, e.g., Lerner & Inouye, "Low copy number plasmids regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability," Nucl. Acids Res. 18(15): 4631 (1990), which was previously submitted). One of ordinary skill in the art would have recognized that plasmid DNA in general constitutes a small fraction of DNA in the host cell and that the situation would be exacerbated where the plasmid copy number is low. In such a situation, efficient retention and recovery would be important, while those of ordinary skill in the art would have considered removal of RNA and genomic DNA to have been a usual step during isolation procedures (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2^{nd} ed.), Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1989), pp. 1.1-1.11, 1.21-1.24, and 1.51). (See First Supplemental Declaration, par. 6.)

7. Additionally, as discussed in my previously filed First Supplemental Declaration, Burgoyne demonstrated recovery of previously purified plasmid from FTA® medium, i.e. where all the input DNA was uniformly plasmid DNA and devoid of other macromolecules present in the original bacterial culture.

8. In my previously filed First Supplemental Declaration, I provided the following example of how this mass discrepancy becomes problematic for the recovery of non-purified plasmids in a bacterial culture:

> If 0.1 microgram of purified pUC19 plasmid DNA (the plasmid example used by Burgoyne) was applied to FTA® medium, this would correspond to approximately 3.4×10^{10} molecules on the paper. If 99% (Burgoyne termed "approximately 100%") of the DNA were detected, then approximately 1% or 3.4×10^{8} molecules of plasmid DNA might not be detected. Conversely 0.1 microgram of E. coli DNA from a culture containing a low copy plasmid only corresponds to approximately 2×10^{7} molecules of genomic *E. coli* DNA and the same number of plasmid DNA molecules, which is

more than an order of magnitude lower in target molecules than the purified plasmid scenario. In the context of finite binding capacity of any substrate where the mass of DNA is independent of genome size, ***the 1.9 x10^7 molecules of plasmid DNA which co-purified with the genomic DNA would be below the total number of plasmid DNA inputted on the paper***. [First Supplemental Declaration, par. 9, see also pars. 6-8, 10; all emphasis in original.]

9. After reviewing the Office Action, mailed 26 January 2010, it is my understanding that the Office Action has focused primarily on the above calculation and the discussion of the previously filed First Supplemental Declaration without sufficient consideration of the many points addressed in the previously filed Declaration.

10. In addition, it is my understanding that the Office Action has requested an additional calculation.

11. It should be noted that the concept of a "copy number" is irrelevant in Burgoyne, because the sample in Burgoyne is previously purified pUC19 plasmid DNA (i.e., 100% plasmid DNA) with no other type of DNA.

12. In the above hypothetical calculation (par. 8), I made several assumptions. First, I was required to make an initial assumption. According to Burgoyne, "approximately 100%" of the DNA in the purified pUC19 plasmid DNA was detected. The precise figure was not provided, so I used an estimate of 99% DNA detection, resulting in 1% undetected DNA, which would be equal to 3.4 x 10^8 molecules of undetected plasmid DNA in Burgoyne, as compared with the above hypothetical low copy plasmid in a mixed population in which the low copy plasmid only corresponds to approximately 2 x10^7 molecules of genomic *E. coli* DNA and the same number of plasmid DNA molecules, which is more than an order of magnitude lower in target molecules than the purified plasmid scenario. As a result, ***the 1.9 x10^7 molecules of plasmid DNA which co-purified with the genomic DNA would be below***

the total number of plasmid DNA inputted on the paper. the 1.9 x10^7 molecules of plasmid DNA.

13. Clearly, despite the assumption of 99% DNA detection in the above calculation, the plasmid of interest in a sample having even ten times the number of hypothetical plasmid DNA molecules might still remain undetected.

14. Alternatively, if one uses an estimate of 90% DNA detection, resulting in 10% undetected DNA, which would be equal to 3.4 x 10^9 molecules of undetected plasmid DNA in Burgoyne, for 0.1 μg of 3.4 x 10^9 plasmid might not be detected and for 0.1 μg of genomic DNA containing plasmids ***this would be over two orders of magnitude below the detectability of low and even many higher copy abundance plasmids.***

15. In addition, it was necessary to assume for the sake of the calculation, which was concerned strictly with the amounts of the different types of DNA (i.e., genomic and plasmid), that the two types of DNA will bind and otherwise behave in a ***uniform*** manner when exposed to the matrix or solid medium, whereas it is known in the art that the two types of DNA often behave quite differently, as discussed in the First Supplemental Declaration (paragraph 6) and in above paragraph 6. For example, it is possible that the genomic DNA, which is usually longer and bulkier, might "crowd out" or entangle the plasmid DNA, which is usually smaller. In contrast, the uniform pUC19 plasmid DNA sample of Burgoyne consists **entirely** of plasmid DNA, so the issue would be non-existant.

16. Furthermore, in a prokaryotic cell (e.g., as in the present invention), the DNA mixture would include both genomic DNA and plasmid DNA. In a eukaryotic cell, the DNA mixture would be more complex and would include not only genomic DNA and plasmid DNA, but also mitochondrial DNA and, in some cases, chloroplast DNA. These other types of non-plasmid DNA could likwise compete with the plasmid DNA of interest and further limit the number of "available sites" on the matrix or solid medium. Similarly, any other types of nucleic acid molecules present in the whole cell samples of the present invention (e.g.,

various types of RNA), might also be expected to compete with the plasmid DNA of interest and still further limit the number of "available sites" on the matrix or solid medium. In contrast, the uniform pUC19 plasmid DNA sample of Burgoyne consists **entirely** of plasmid DNA.

17. In addition to other types of nucleic acids in the sample, in the present invention, the plasmid DNA is isolated from whole cells having proteins and other types of macromolecules. These macromolecules would be present on the matrix or solid medium as part of the dried spot sample. Again, in contrast, the uniform pUC19 plasmid DNA sample of Burgoyne consists **entirely** of plasmid DNA.

18. The present invention provides methods of isolating plasmid DNA from a whole cell of far greater complexity than that of the previously purified pUC19 plasmid sample of Burgoyne.

19. Therefore, the teachings of Rogers, Burgoyne, and Kahn, either alone or in combination, would not have suggested the present invention to one of ordinary skill in the art with any reasonable expectation of success.

20. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 25 March 2010

Walter King

Walter King, **Ph.D.**